Can dietary nitrate supplements improve tolerance to hypoxia? 1AO1

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This article is a review of the rationale and methodology of a translational study conducted at altitude investigating the potential role of nitrate supplements to improve tolerance to hypoxia, and a discussion of the applicability of the findings to intensive care medicine.

Keywords: *hypoxia; diet; nitrate supplements*

Introduction

Tissue hypoxia is a problem which afflicts a significant proportion of patients admitted to intensive care units. Left unchecked, this insult leads inevitably to organ dysfunction and a high rate of mortality. Indeed, the survival of many critically unwell patients is determined by their capacity to tolerate oxygen insufficiency. Predicting an individual patient's response to an hypoxic insult is difficult and it has become clear that a better understanding of human physiological responses to hypoxia is called for in order to guide therapies that might confer a survival advantage to these patients.

This improved understanding has been occasioned by the study of populations both permanently living at, and temporarily travelling to, high altitude. At altitude, the human body is subjected to hypobaric hypoxia concomitant with a decrease in the partial pressure of oxygen. The hypoxaemia and tissue hypoxia which results from a lowlander ascending to high altitude has parallels with the hypoxia which afflicts many patients admitted to intensive care units.

More recently, novel therapeutic avenues of relevance to intensive care medicine have come to light from the study of the physiological adaptations of Tibetan highlanders. This group outperforms lowlanders acclimatised to high altitude for reasons that have long eluded researchers. The observation that this group have significantly higher concentrations of circulating markers of nitric oxide (NO) than those not accustomed to living at high altitude has raised the possibility that NO may have a role in their remarkable hypoxic adaptation. This has fuelled an interest in whether therapeutic modulation of bodily NO might confer similar benefits to non-Tibetan climbers, or indeed to critically unwell patients in intensive care faced with a similar hypoxic insult. This article aims to set out the rationale for the Margherita Hut research expedition of 2010, in which the author participated, and provide insights into the practical conduct of the study, before concluding with a discussion of the strengths and limitations of translational research of this nature. The results of the study itself are not yet published and will not be considered here.

Background

Hypoxia and why it matters

Intensivists are responsible for the care of critically ill patients, where one of their prime concerns is maintaining adequate tissue oxygenation. Hypoxia, whether localised to a particular tissue or generalised throughout the body, is an almost ubiquitous feature of critical illness.[1] Tissue hypoxia results either from oxygen demand outstripping supply, or decreased ability of cells to use available oxygen. When persistent, it leads to disruption of cellular metabolism and ultimately hypoxic injury and organ dysfunction, with some tissues being more sensitive than others to a hypoxic insult.[2] At a cellular level, sustained hypoxia leads to decreased mitochondrial oxidative phosphorylation and depletion of ATP, offset to some extent by anaerobic glycolysis. However, this is unsustainable as cellular glycogen stores are progressively run down and intracellular pH falls. Subsequently, critical depletion of ATP below 5-10% of normal levels sets in motion a series of adverse cellular events culminating in energy-dependent ion pump failures, increased cytosolic calcium concentration, activation of deleterious enzymes and increased mitochondrial permeability – eventually leading to irreversible damage and cell death.[3]

More oxygen is not always the answer

To avoid the induction of this process, or at least to reverse the early stages of hypoxic cellular dysfunction, the default thinking has – seemingly reasonably – been to attempt to improve oxygen delivery to these hypoxic tissues. Delivery of oxygen is limited by convective capacity (bulk movement of oxygen in air or blood, driven by active, energy-consuming processes generating flow in the airways and circulation) and by diffusive capacity (passive movement of oxygen down its concentration gradient across tissue barriers).[2] Optimising convective oxygen delivery early in critical illness appears to be beneficial, yet rather surprisingly some evidence suggests that using the same strategy to restore oxygen delivery to pre-morbid levels in established critical illness can be harmful to patients.[4,5] It is now recognised that 'downstream' factors, including oxygen distribution via the microcirculation and

diffusive transport processes occurring later in the oxygen cascade, are more significant determinants of cellular hypoxia and subsequent organ failure than are limitations in convective delivery of oxygen.[2] It has been noted that microcirculatory dysfunction, characterised by decreased recruitment and perfusion of the smallest capillaries (<100 µm in diameter) where oxygen release to the tissues takes place, is practically a universal feature in septic shock, contributing to continued regional hypoxia and reduced oxygen extraction despite the restoration of convective oxygen delivery variables.[6]

That attempts to increase convective oxygen delivery have been unsuccessful in improving survival in critical illness may reflect a misunderstanding of what limits oxygen supply to hypoxic tissues, with excessive focus on the operation of the macrocirculation and neglect of the microcirculation. The fact that augmenting convective oxygen delivery may even be detrimental to survival in established critical illness might be explained by the fact that hypoxia-induced (and hypoxia-dependent) adaptive mechanisms have come into play that increase cellular metabolic efficiency, thereby reducing oxygen needs, and that these adaptive mechanisms are vulnerable to disruption when sudden attempts are made to restore normoxia.

It is becoming increasingly clear that crude efforts to supply more oxygen to hypoxic tissues may fail because they overlook the subtle means by which adaptations to hypoxia are made, which may involve modulation of oxygen delivery via the microcirculation and of oxygen utilisation by cellular machinery under hypoxic stress. These adaptations are steadily being elucidated, and a better understanding of them may engender a paradigm shift in the treatment of hypoxia.

Lessons from high altitude

Studying hypoxia among the critically ill is fraught with difficulty. Patients typically have very variable pre-admission fitness characteristics, and a diverse range of problems often compounded by significant co-morbidities.[7] In these heterogeneous circumstances, the potential for the influence of confounding variables is intimidating. To overcome these difficulties, some researchers have sought to investigate hypoxic adaptation in relatively homogeneous groups of healthy volunteers ascending to altitude. This approach permits a more reliable characterisation of the effects of hypoxia on human physiology, while providing the opportunity to investigate the efficacy of therapeutic interventions in a group more amenable to statistical analysis than patients.

The first premise underpinning research of this nature is that the hypobaric hypoxia induced by ascent to altitude provides an accurate simulation of the hypoxia induced by pathology among critically unwell patients, producing a similar physiological response. This seems probable at present; the physiological response to exposure to altitude has marked parallels to the response seen in critical illness where hypoxia is a feature. Early haematological and cardiorespiratory responses to ascent favour increased convective oxygen delivery, suggesting that this is adaptive, and achieving a similar effect therapeutically in early critical illness also confers benefit as previously described. Yet after more prolonged exposure to hypobaric hypoxia, a sudden increase in oxygen delivery enabled by breathing supplementary oxygen does not boost oxygen consumption back to maximal sea level values and worsens microcirculatory dysfunction, perhaps in keeping with the finding that augmenting oxygen delivery later in critical illness can worsen outcomes.[7,8] Moreover, microcirculatory dysfunction seems to be a feature common to both critical illness and ascent to altitude.[8]

The second key premise is that similar explanations may account for the observation that some critically ill patients survive hypoxia while others do not and the parallel observation that some people ascending to high altitude adapt or acclimatise better than others. In both cases it is difficult to predict how a particular individual will fare given their baseline characteristics.[5,7] Indeed it has simultaneously puzzled investigators in the fields of intensive care medicine and high-altitude physiology that cardiovascular fitness appears to be a poor predictor of survival and performance respectively (as Martin writes: 'high altitude heroes are rarely athletes at sea level').[5] In each case the implication borne out time after time is that inter-individual variations in hypoxic adaptation are inadequately explained by differences in oxygen supply alone.[5] It might be that filling the gaps in our understanding of the variability in human performance at altitude will also improve prognostication in intensive care medicine.

Recent papers are beginning to yield results which are elucidating possible mechanisms of adaptation to hypoxia,[9] as well as identifing genetic determinants of hypoxic performance.[10,11] These insights are paving the way for the exploration of novel therapeutic approaches to hypoxia. This article traces the emergence of a new paradigm for understanding hypoxic adaptation in humans centred on the role of nitric oxide (NO). It will discuss the Margherita Hut research expedition of 2010, the goal of which was to assess the efficacy of a new intervention aimed at improving tolerance to hypoxia.

The role of nitrates, nitrites and NO

Nitric oxide as a mediator of adaptation to chronic hypoxia

Nitric oxide (NO), well-known as a short-acting vasodilator, has established itself in the limelight in hypoxia research. A role for NO in hypoxic adaptation was first recognised after it was observed that concentrations of exhaled NO are substantially higher among two high-altitude-dwelling populations under chronic hypoxic stress – Tibetan highlanders living at 4,200 m and Bolivian Aymara living at 3,900 m – than among lowland populations.[12] It is well-established both empirically and anecdotally that such long-term highland dwelling populations have a superior ability to tolerate hypoxia – as judged by better physical performance at altitude – than does the general populace, so these findings raised the prospect that NO might be involved in a pulmonary adaptation to chronic hypoxia.[13,14] Subsequent investigations found blood markers of endogenous NO production to be systemically elevated more than ten-fold in Tibetan highlanders (in excess of levels typically encountered in cases of septic shock), implying a likely wider role for NO beyond the lungs,

and the prospect of NO being involved in hypoxic adaptation gained further momentum.[15] The effects of this additional NO load on the dynamics of the peripheral circulation are marked, as indicated by forearm plethysmography measurements which showed that Tibetan highlanders had double the forearm blood flow when compared to a sample of sea-level residents.[15] This is suggestive of a unique approach to chronic hypoxia centred on vascular rather than haematological or pulmonary factors, enabling the Tibetans to achieve a rate of oxygen delivery ($\dot{D}O_2$) to the forearm capillary beds, more than twice that of sea-level based controls, despite having on average a lower arterial oxygen content.[15]

Generation of NO

Historically, all NO was presumed to originate from the oxidation of the amino acid, L-arginine by NO synthases (NOSs) in an oxygen-dependent process.[16] However, a great deal more about the secret life of NO and its closely-related precursor molecules nitrite (NO_2^-) and nitrate (NO_3^-) has been unravelled. In particular, an alternative NOS-independent pathway of NO generation has been recognised which becomes important during hypoxia. Moreover, this newly uncovered method of NO synthesis provides pragmatic opportunities for therapeutic augmentation.

This alternative pathway of NO generation came to light following a series of discoveries, beginning with the observation that NO is generated at high concentrations in the stomach.[16,17] It was deduced that this was derived from dietary nitrate in what constituted an alternative pathway for NO generation not requiring L-arginine or NOS. Dietary nitrate is absorbed in the gut, and with a 5-8-hour half-life circulates in the plasma and is concentrated 10-20-fold in the salivary glands.[16] Oral commensal bacteria reduce the nitrate – which is a biologically inert molecule – to nitrite, which is then regularly swallowed. Once in the stomach, the low pH protonates a small proportion of the swallowed nitrite to form nitrous acid (HNO_2), which subsequently decomposes to form NO.[16] NO has a very short half-life of a few seconds and therefore exerts only very localised effects. The majority of the swallowed nitrite escapes protonation and enters the systemic circulation, where its 20-minute half-life enables it to reach distal tissues.

Nitrite can be reduced to NO outside the stomach by a number of different catabolic enzymatic pathways:[16] by xanthine oxidoreductase, by elements of the mitochondrial respiratory chain, by myoglobin in hypoxic myocytes, by haemoglobin and by a peculiar form of erythrocyte membrane-associated NOS.[17-19] Significantly, the activity of all these pathways becomes maximal at low oxygen tension.[16]

In summary, the entero-salivary circulation of nitrogen oxides is a feature of normal physiology which provides the systemic circulation with a constant supply of nitrite. Its continuous operation puts nitrite at the disposal of hypoxic tissues, where it fuels an alternative pathway of NO generation. Long-lived nitrate has been likened to a pro-drug, in that it provides a stable, slow-release substrate for nitrite production.[18] Furthermore, ingesting more nitrate does seem to cause a corresponding increase in plasma nitrite levels,[20] raising the possibility that dietary supplementation with nitrates might achieve levels of NO availability comparable to those in high altitude-dwelling Tibetans, where generations of adaptation to an hypoxic environment have likely favoured an exaggeration of these apparently ubiquitous physiological responses.

Effect of increasing dietary nitrate intake

Nitrates have been a source of controversy in public health debates. They have been associated with gastric cancer, as well as blue-baby syndrome (methaemoglobinaemia) and as a result have acquired a reputation for being hazardous.[17,18] This increasingly seems to have been premature and misleading, as nitrate seems to have a pivotal role in human physiology quite apart from its possible role in adaptation to chronic hypoxic challenge. It is thought to be a principal contributor to the health promoting effects of the Mediterranean diet, be cytoprotective in ischaemia-reperfusion injury, reduce blood pressure without any reduction in efficacy from the emergence of tolerance and have antimicrobial properties in the stomach – and all these achieved at relatively low concentrations.[17]

The quantity of nitrate required to produce the plasma levels observed in Tibetan highlanders is readily obtainable from a diet rich in nitrate-containing vegetables, notably beetroot, spinach and lettuce.[18] Investigations comparing the effects of regular consumption of suitable quantities of nitrate-rich vegetables and comparable nitrate doses administered regularly in isolation via a sodium nitrate pill, demonstrate comparable efficacy, indicating that nitrate is indeed the active ingredient in these vegetables. These studies open the possibility of a simple intervention to boost hypoxic tolerance, with the potential for rapid adoption of dietary supplementation without the need for drawn-out, prohibitively expensive pharmaceutical trials. At a time when there is acute pressure to control ballooning health expenditure in the UK, the refreshing prospect of a cheap and widely useful intervention in an arena of medicine traditionally synonymous with resource-intensiveness surely warrants further investigation.

Mechanism of action of NO in mitochondria

As we have seen, the Tibetan physiological approach to chronic hypoxia is unmatched in effectiveness by the acclimatisation responses of lowlanders ascending to altitude. Tibetans appear able to maintain higher work rates at reduced oxygen cost compared to lowlanders.[21] This has always been suggestive of better efficiency of energy production, though the mechanism for this was unknown. Recent work has highlighted the fact that dietary nitrate supplementation seems able to reduce the oxygen cost of exercise, for the first time linking the observation of high levels of circulating derivatives of NO in Tibetans with their apparently superior metabolic efficiency.[22-24]

This further role for NO may help account for the discrepancy in hypoxic performance between NO-replete Tibetans and acclimatised, but relatively NO-depleted, lowlanders. The crucial differentiating factor may be the way that these groups are able to utilise available oxygen. More work is needed to elucidate the precise mechanisms by which NO acts to improve hypoxic tolerance, though some progress has been made. In a recent study which allocated participants

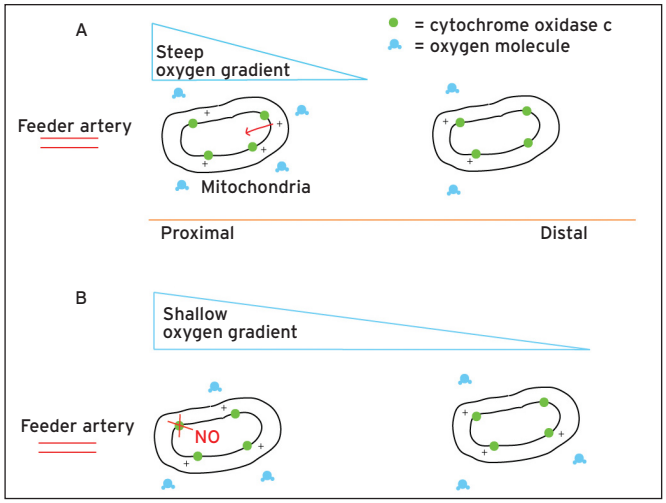

Figure 1 Schematic illustrating how NO may act to preferentially inhibit the proton pumping activity of cytochrome oxidase c in mitochondria close to a tissue's arterial supply, thereby maintaining lower inner mitochondrial membrane potentials and contributing to reduced proton leakage. **A** shows situation with poor NO availability; **B** shows situation with increased NO availability in which there is more even distribution of available oxygen among mitochondria, reduced proton leakage, and consequently increased ATP production per unit oxygen delivered to the tissue.

to either nitrate supplementation (using a dose equivalent to 100-300 g of nitrate-rich vegetables) or a placebo, the intervention group demonstrated a significant reduction in $\dot{V}O_2max$ while maintaining work performance at maximal exercise.[22] These findings question the presumption of a linear relationship between $\dot{V}O_2$ and work rate, implying that metabolic efficiency – the relationship between oxygen uptake and work rate – is, to a degree, modifiable by nitrate administration. Nitrate ultimately mediates the step-up in metabolic efficiency observed in respiring cells under conditions of physiological hypoxia induced by exercise.

A range of possibilities compete to provide the explanation for this.[23] While the findings of the study relate specifically to muscular work, it is tempting to speculate that increased NO availability might increase the oxygen efficiency of such work by decreasing the oxygen cost of ATP production during oxidative phosphorylation, conferring adaptive benefit to all hypoxic tissues.

A theoretical basis for NO reducing the oxygen cost of ATP synthesis in hypoxic tissue has been described as follows (**Figure 1**): NO is known to compete with oxygen to bind to cytochrome c oxidase, the terminal electron acceptor in the mitochondrial electron transport chain.[18] In hypoxic tissue, NO is generated preferentially when the supply of its substrate, nitrite, is most abundant, which will be nearest to the feeder artery. The feeder artery is also the source of the limited amount of incoming oxygen. Competitive inhibition of cytochrome oxidase c by NO spares oxygen and reduces the inner mitochondrial membrane (IMM) potential. With this electrochemical gradient reduced, the proton motive force is reduced, meaning less ATP generation occurs in these proximal mitochondria. Crucially however, there is also reduced H^+ leak back across the IMM into the mitochondrial matrix from the intermembrane space. Proton leakage can represent a significant non-ATP-generating use of oxygen, accounting for some 15-20% of resting oxygen consumption.[22] In contrast, cells distal to the feeder artery inevitably have a poorer supply of nitrites and therefore correspondingly less NO synthesis and a lesser degree of cytochrome oxidase c inhibition. They could therefore be able to use the oxygen spared from more proximally located mitochondria to generate ATP. The effect is one of extending the oxygen gradient through hypoxic tissue, with oxygen being distributed more evenly over a greater population of mitochondria. If NO acts to prevent the formation of steep IMM electrochemical gradients, and in so doing reduces non-ATP producing proton leakage, it may render mitochondria more efficient energy converters.[22,25] This is in keeping with the concept in thermodynamics that maximum efficiency and maximum power cannot be achieved simultaneously.[26]

Alternative proposed mechanisms to explain the empirical findings include the suggestion that the effect results from decreased slippage at mitochondrial cytochrome oxidase c proton pumps in the presence of NO,[27] or from recruitment of more oxygen efficient substrates for oxidative phosphorylation. Alternatively decreased oxygen utilisation during hypoxic stress may allow efficiency savings while ATP generation remains constant: non-essential ATP-consuming cellular activity may be down-regulated during hypoxic stress, or the ATP cost of cellular activities – such as the events leading to muscular force production – may be reduced.[1,23] However, recent research has indicated a further mechanism through which nitrate can induce an intrinsic improvement in skeletal muscle mitochondrial efficiency by decreasing the expression of a specific mitochondrial protein associated with proton leakage called ANT.[26] This increasingly seems like the primary means by which nitrate exerts its effect.

In summary: rationale for study

NO is probably involved in at least two aspects of adaptation to hypoxia. On the supply side it enhances peripheral blood flow and oxygen delivery to hypoxic tissues principally via beneficial effects on the microcirculation. A second and intriguing manner in which adaptation to hypoxia may be enhanced by increased NO availability focuses on its role in decreasing oxygen demand. Mechanisms have been proposed by which NO might enhance the metabolic efficiency of mitochondria in hypoxic cells, allowing hypoxia to be more readily tolerated. Considered together, the proposed dual effect of NO at the microcirculation-mitochondrial unit (**Figure 2**) may represent an effective means of mounting a physiological response to hypoxia which maximises the efficiency of consumption of available oxygen. While further investigation to clarify the precise mechanism(s) in play and their relative contributions is undoubtedly required, the stage is now set for upstream manipulation of NO availability in distal tissues in order to improve hypoxic tolerance by increasing dietary nitrate input.

The Margherita Hut study set out to examine whether dietary nitrate supplementation sufficient to achieve comparable circulating levels to those in Tibetans could improve hypobaric hypoxic performance, which might

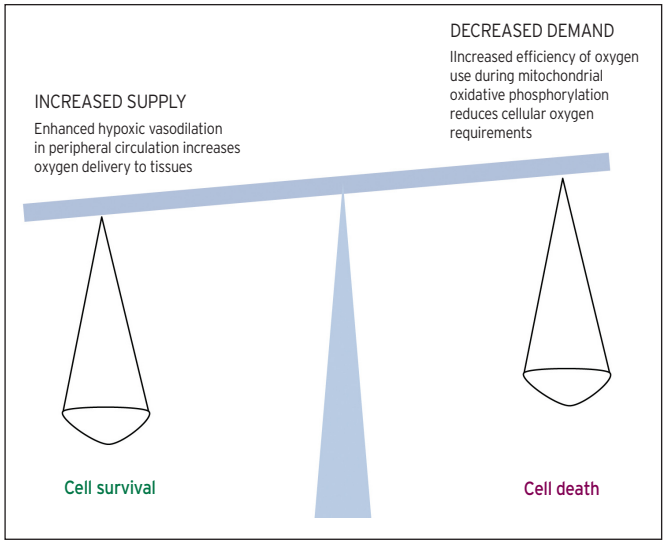

Figure 2 The proposed dual actions of NO on oxygen supply and demand which act to tip the balance of hypoxic status in favour of cell survival.

subsequently translate into a therapeutic role for nitrates in a clinical setting.

Study outline

Design and setting

The study was a double-blind, randomised controlled trial to assess the effect of dietary nitrate supplementation on human adaptation to hypobaric (altitude-induced) hypoxia. There were two phases to the study: sea-level testing in London and then a repeat of the same testing protocols at a laboratory established at an altitude of 4,559 m on Monte Rosa in the Italian Alps. At this altitude, the partial pressure of oxygen is approximately 12 kPa, just over half the sea-level figure. The altitude phase was undertaken over 14 days, including a step-wise ascent to 4,559 m over four days. This pattern of ascent profile was necessary to ensure the safety of the research team: too rapid an ascent can precipitate acute mountain sickness, high altitude pulmonary oedema (HAPE), high altitude cerebral oedema (HACE) and other serious complications. Besides the need to minimise these risks, it seems reasonable to suggest that a four-day ascent profile might approximately simulate the typical deterioration of a sick patient towards worsening hypoxaemia. Hypoxic performance was assessed by cardiopulmonary exercise testing, and it was hypothesised that the intervention group would see less of a decline in their performance at altitude relative to sea-level than would the control (placebo) group.

Subject selection

Forty healthy adult subjects were selected to take part. All subjects were screened for adverse responses upon previous ascent to high altitude. The size of the study was a compromise between practical limitations imposed by the unorthodox laboratory setting and recruiting enough subjects to power the study sufficiently to detect a modest difference in measures of hypoxic adaptation between the nitrate intervention and placebo groups. To minimise the confounding effects of any prior acclimatisation on the results, all subjects were instructed to avoid ascent to altitudes above 2,000 m in the eight weeks prior to commencing the study. The subjects of the study were also the investigators and responsible for carrying out the research protocols, having had suitable training and practice at sea level; this was a necessity given the number of tests concurrently underway.

Dietary supplementation

Half the subjects were randomly allocated to the nitrate intervention group, and the remainder to a placebo control group. The nitrate intervention constituted sodium nitrate at a dose of 0.1 mmol/kg/day, mixed with fruit juice and administered three times daily at regular intervals. Each nitrate dose corresponded approximately to 100-300 g of nitrate-rich vegetables. The controls were given a plain fruit juice drink flavoured and coloured to mimic the intervention. The intervention drink was a prototype therapeutic intervention potentially appropriate for hospital use.

Cardiopulmonary exercise testing and basal metabolic rate

Cardiopulmonary exercise (CPX) testing was conducted on stationary exercise bikes following a period of six hours of fasting and at least 24 hours abstention from strenuous exercise, caffeine and alcohol (all of which can modify physical performance). Unlike using treadmills, CPX testing on stationary bikes has previously been shown to produce more consistent results, with less of a tendency for experienced users to use a more efficient technique to achieve greater work rates for a given oxygen consumption. Throughout CPX testing, a sophisticated mask analysis system was used to record the changing turnover of ventilator gases in the subject's exhaled breath. Two automated tests were done at each altitude: a 30 minute bout of moderate intensity cycling to assess sub-maximal oxygen consumption and a ramp ($\dot{V}O_2$max) test up to exhaustion, which varied in duration between subjects. The same breath-analysing instruments were used to assess basal oxygen uptake with subjects kept supine and warm. Data from this study should act to either corroborate or contradict the expectation that the oxygen costs of basal metabolism, moderate exercise and maximal exercise are all reduced by nitrate supplementation under conditions of ambient hypoxia, and that this is reflective of an increase in metabolic efficiency facilitated by nitrate supplementation.

Strengths and limitations of translational research at high altitude

Practical benefits of using mountaineers in lieu of the critically ill

Research of this nature can accelerate the process of translating progress in laboratory science into actual tangible benefits to sick patients. This study was able to circumvent the drawn-out process of undertaking a clinical trial, which can be financially prohibitive and time-consuming. Such an approach naturally has advantages and limitations. The practical and ethical difficulties in coordinating a study testing a dietary nitrate supplement among its target users who are critically ill are

significant. This is especially so given the need to consider the variety of comorbidities that may be contributing to the circumstances of different critically hypoxic patients in the hospital setting, as well as the lack of information about their physiological status prior to admission. Gaining consent from these patients may also be fraught with complexity. This study avoided these problems by taking a relatively homogeneous group of willing healthy volunteers, whose baseline physiological performances could be objectively assessed at sea level, before exposing them to a hypoxic stress of identical severity and retesting their physiological performance. Hence the dependent variable of prime concern (adaptation to hypoxia) could be more readily isolated.

Representativeness of the study group

The subjects in this study were healthy volunteers, not critically ill. Moreover, the study group featured a disproportionate number of accomplished mountaineers who were naturally attracted to participate in a study of this kind. Individuals who climb mountains and then go on to do so again are presumably relatively adept at adapting to hypoxia, since if they were not they would tend to feel unwell much of their time, not achieve their mountaineering goals, be discouraged and probably – if they have any sense – take up an alternative pastime. Therefore, to a significant degree, the study group was self-selecting. What is more, the average ability of the largely young and fit study cohort to recruit NO via the alternative pathway may be better than that of someone whose physiological reserve is compromised by ill health

However, there is no suggestion that any findings from this experiment can or should be applied directly and uncritically to sick patients. Rather, the investigation was conceived as a pilot study. This was more easily achieved using willing healthy volunteers in an environment of hypobaric (exogenous) hypoxia rather than hypoxia resulting from endogenous disease processes. Notwithstanding the differences between these two groups, this translational study has great value as a proof of principle, which may spur on further investigation in this field and accelerate the process of getting an idea from 'bench to bedside' if it can be proven to have potential benefit.

The future

Elucidating the relative contributions of the proposed mechanisms involved in reducing the oxygen cost of exercise is a research priority. This crucial information will help researchers to understand the full potential and possible limitations of NO as a therapeutic agent in chronic hypoxia and assist in the identification of other prospective targets for therapeutic interventions which may benefit critically hypoxic patients under the care of intensivists.

NO is thought to have a number of physiological roles in the stomach including mucosal protection and a bactericidal action.[8] These may be interrupted by decreased availability of substrates for NO synthesis resulting from a number of common practices in intensive care medicine, for example administration of antibiotics may preclude the bacterial reduction of salivary nitrate into nitrite, reducing downstream NO availability. Intubation prevents swallowing of saliva, breaking the entero-salivary cycling of nitrates and nitrites with the same consequences. The use of nitrate-deficient enteral and parenteral feeding formulas may further exacerbate the scarcity of all components of the nitrate-nitrite-NO axis.[8] The corollary is that current practice in intensive care may unwittingly act to the detriment of the normal mechanisms of adaptation to chronic hypoxia. Clearly there is a striking need for further studies to assess the clinical significance of these potentially negative effects.

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This article is based on an essay which was the winner of the 2011 ICS Medical Student Essay prize

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Commentary

Steve Dauncey's winning essay in the ICS Medical Student Essay Competition is a beautifully written exposition of a developing area of physiology with direct relevance to critical illness.[1] Through the description of a recent experiment at high altitude, the essay explores developments in our understanding of the effects and metabolism of nitrogen oxides (nitric oxide (NO), nitrate, nitrite) and the potential relevance of these insights to the pathophysiology of critical illness.[2] This research area is based on the premise that exploring inter-individual differences in adaptation to hypoxia in healthy individuals at altitude may lead to novel insights in the understanding of critical illness,[3] and the winning essay comprehensively explores the strengths and weaknesses of this paradigm.

The classical pathway of nitric oxide (NO) generation involves oxidation of L-arginine catalysed by the enzyme NO-synthase. More recent attention has focused on reductive pathways of NO generation from nitrite (NO_2^-) and nitrate (NO_3^-). This is of particular interest to those involved with the care of the critically ill for two reasons: reductive NO generation may be preferentially disinhibited under conditions of hypoxia[2] and supplementation of nitrite/nitrate is readily achieved through dietary or pharmacological interventions.[4]

High levels of NO products have been demonstrated in Tibetan highlanders in association with elevated forearm blood flow.[5] Our group has shown elevated levels of nitrogen oxides in lowlanders ascending to altitude coupled with an association with sublingual microcirculatory blood flow.[6] Oxygen dependent changes in nitrogen oxide metabolism modulating microcirculatory oxygen convection[6] and cellular oxygen metabolism[7] provide plausible mechanisms for acclimatisation to hypoxia at high altitude.

So could similar mechanisms be important in the pathophysiology of critical illness? Despite evoking significant physiological responses, NO supplementation in ARDS[8] and NO-synthase inhibition in sepsis[9] did not improve clinical outcomes. However, recent studies of NO donors in sepsis have shown promise.[10] The concept that a single intervention will benefit all patients with a particular condition is increasingly recognised as naive. This is particularly true in critical illness where the treated 'conditions' are based on clinical diagnostic criteria that do not consistently map onto well-defined pathophysiological phenotypes.[10] Looking forward, novel medical interventions will be targeted to specific patient groups through the use of high-quality phenotyping coupled with genomic signatures.

The 2010 'Xtreme-Alps' study, led by Dr Dan Martin (Director of UCL CASE Medicine), builds on previous work by the Xtreme Everest group (www.xtreme-everest.co.uk) including the Caudwell Xtreme Everest expedition in 2007. In contrast with the group's previous studies that have been predominantly observational, this was a randomised controlled trial (RCT) of a nitrite-based intervention in healthy volunteers ascending to altitude. This brings the group to the threshold of clinical studies in patients, for which funding has recently been secured from the National Institute of Health Research.

Steve Dauncey and others like him, represent the face of a new generation of investigators drawn to this research area. Xtreme Everest has developed into a multi-institutional (UCL, University of Southampton, Duke University) research consortium with three parallel work-streams: clinical, field and laboratory/chamber studies. A central plank is the development of a 'stratified medicine' approach to the management of critically ill patients, with treatment guided by biomarkers derived from the healthy volunteers studies. While the clinical benefit of this approach remains uncertain, the degree of uncertainty is decreasing year-on-year.

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